REMARKS

The present invention is predicated on the discovery that gene expression driven by a heterologous promoter in an expression vector can be tightly regulated by incorporating a silencer-inducible region including a silencer element and an inducible element in the vector. Transcription of the gene is effectively inhibited when a silencer protein is bound to the silencer element. This silencing is reversed in the presence of a signal that causes a transcription factor to both engage the inducible element and, by steric hindrance, displace binding of the silencer protein to the silencer element.

Prior to the invention, it was suggested that silencer elements could be used to downregulate transcription of a gene in a vector. Because the duration of such silencing was observed to last a lifetime in animals, the binding of a silencer element to a silencer protein was believed to be difficult to disrupt. Thus, the discovery that such silencing could indeed be reversed by placing an inducible element in close proximity to the silencer element such that engagement of the former by a transcription factor disrupts the silencer protein-silencer element interaction was surprising and unexpected. When used in combination with a tissue-specific promoter, the invention thereby allows tissue-specific expression of a gene in an almost "on and off" fashion depending on the presence of a particular signal. This system is therefore particularly advantageous for treating conditions (e.g., ischemia) where even basal level expression of a therapeutic gene could be deleterious to healthy tissue.

Status of the Application

Claims 1-27 and 42-60 were pending in the application at the time the Office Action was mailed. Claims 2, 3, 10, 13-17, 20, 54, 55 and 59 were withdrawn from consideration, and claims 1, 4-9, 11, 12, 18, 19, 21-27, 42-53, 56-58 and 60 were rejected. No claims were allowed.

By this amendment, claim 1 has been revised, no claims have been added, and claims 22 and 50 have been canceled. Therefore, claims 1, 4-9, 11, 12, 18, 19, 21, 23-27, 42-49, 51-53, 56-58 and 60 are now before the examiner for consideration.

Rejection Under 35 U.S.C. 102

Claims 1, 4-6, 11-12, 19, 21, 23-24, 26-27, 42, and 60 were rejected under 35 U.S.C. 102(b) as being anticipated by Webster et al. (WO 96/20276). In particular, the Office Action states that:

> Webster et al. teach expression vectors comprising a hypoxia response element (HRE) from the erythropoietin gene (reads on SEQ ID NO:1) operatively linked to a tissue-specific promoter, such as a cardiac-specific promoter (pages 5-6 and 12). It is taught that the expression vector may be a plasmid, a replication-defective adenovirus vector, retrovirus vector, or the like (pages 6 and 33). Termination, polyadenylation, and other sequences for effective expression in cells may be in the expression vectors (page 20). The expression vector containing a luciferase reporter gene is also taught (pages 32 and 44). This reference also teaches that: "A further desirable characteristics of promoters useful in the present invention is that they possess relatively low activity in the absence of activated hypoxia-regulated enhancer elements, even in the target tissues. One means of achieving this is to select promoters of genes encoding proteins that have a relatively low turnover rate in adult tissue, such as the actin and alpha-MHC promoters described herein. Another means is to use "silencer" elements, which suppress the activity of a selected promoter in the absence of hypoxia." (page 12). This reads on adding a heterologous silencer element to the constructs taught by the reference in order to reduce expression from the promoter in the absence of the induction by hypoxia (i.e., forming a silencer-inducible region as claimed).

As the examiner noted, at page 12, WO 96/20276 suggests that silencer elements can be used to regulate transcription of a gene by suppressing the activity of a promoter operably linked to the gene. WO 96/20276, however, does not explicitly describe any working or even hypothetical examples of vectors incorporating such a silencer system. Moreover, aside from the one sentence statement quoted in the Office Action, WO 96/20276, provided no other teaching on how this might actually be implemented in an expression vector.

Amended claim 1, presented herein, from which all the remaining claims depend includes tw elements neither expressly or inherently disclosed in WO 96/20276: (a) "at

least one of said silencer elements and one of said conditionally inducible elements are arranged within 500 nucleotides of each other" and (b) that "wherein silencing of expression of said at least one nucleotide sequence from said expression vector is reversed in the presence of an inducing condition." As WO 96/20276 does not teach at least these two elements of the currently claimed expression vectors, withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. 103

> Claims 1, 4-9, 11-12, 18-19, 21-27, 42-53, 56-58, and 60 were rejected under 35 U.S.C. 103(a) as being unpatentable over Webster et al. (WO 96/20276) in view of Li et al. (applicant reference KR). As indicated above, amended claim 1 recites at least two limitations not expressly or inherently disclosed in WO 96/20276: (a) "at least one of said silencer elements and one of said conditionally inducible elements are arranged within 500 nucleotides of each other" and (b) that "wherein silencing of expression of said at least one nucleotide sequence from said expression vector is reversed in the presence of an inducing condition." Li et al. also does not teach either of these limitations.

As mentioned above, WO 96/20276 merely suggests, in a single sentence, that silencer elements can be used to regulate transcription of a gene by suppressing the activity of a promoter operably linked to the gene. Li et al. describes the characterization of a silencer element from the human synapsin gene that is capable of conferring repression on a heterologous promoter in non-neuronal cells. Neither WO 96/20276 or Li et al. teach an expression vector featuring a silencer element arranged within 500 nucleotides of a conditionally inducible element or an expression vector that is reversibly inducible.

As evidenced by both WO 96/20276 and Li et al., at the time of the invention, it was known that silencer elements could repress the expression of a gene under the control of a heterologous promoter. However, what was not known at this time was that this silenced gene expression could be reversed. If anything, the evidence at that time

supported that it would be quite difficult to reverse such gene silencing, e.g., because silencer elements were known to repress gene expression in a tissue-specific manner for the lifetime of the organism (neuronal-specific genes are never expressed in non-neuronal tissues because they are "silenced"), it was thought that that silencer proteins bind strongly and permanently to their target DNA elements. Thus the discovery that such silencing could be reversed (e.g., by steric hindrance from a transcription factor) was an unexpected finding that was not suggested by WO 96/20276, Li et al., nor within the knowledge of one of ordinary skill in the art at the time the invention was made. Accordingly, as the combination of WO 96/20276 and Li et al. does not teach all the elements of the claimed invention nor suggest the presently claimed invention to one of ordinary skill in the art at the time the invention was made, withdrawal of this rejection is respectfully requested.

Double Patenting

The office action objected to Claim 50 under 37 CFR 1.75 as being a substantial duplicate of claim 49. Claim 50 has herewith been canceled.

Conclusion

The currently pending claims before the examiner are supported throughout the specification and are patentable over the prior art. No new matter has been added. This application is now in full condition for allowance, and such action is respectfully requested.

Accompanying this reply is a retroactive petition for a three month extension of time. The Commissioner is hereby authorized to charge any underpayment or credit any overpayment of fees under 37 CFR 1.16 or 1.17 as required by this paper to Deposit Account 50-3110.

The examiner is cordially invited to call the undersigned if clarification is needed on any matter within this amendment, or if the examiner believes a telephone interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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